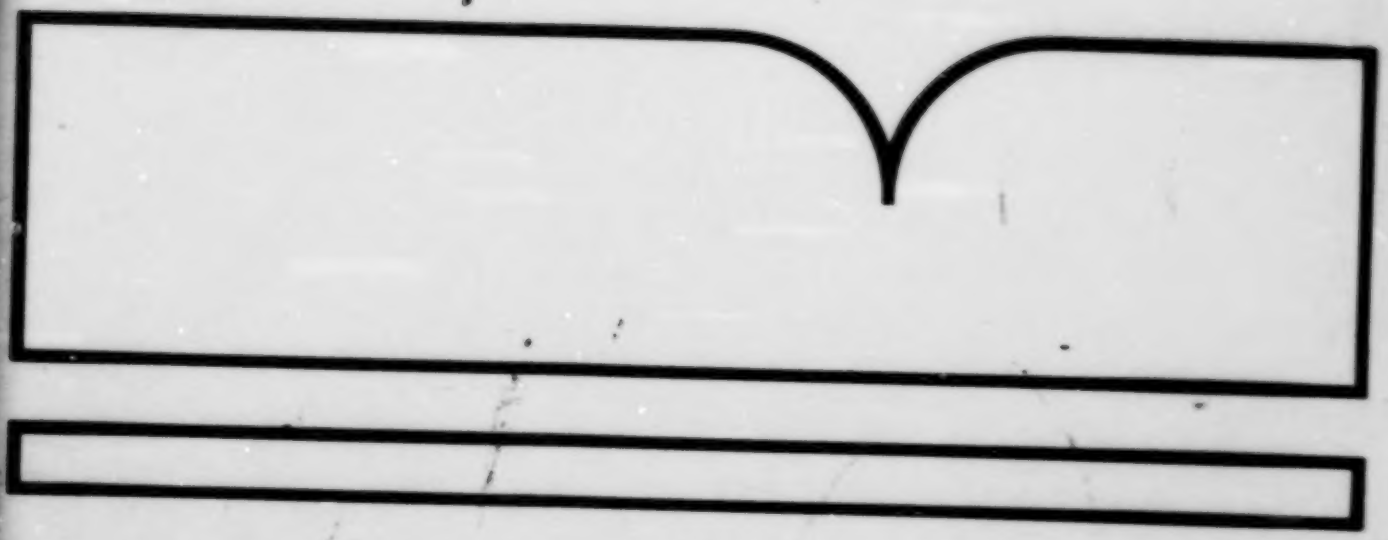
PB85-144103

Biological Methods for Determining Toxicity of Contaminated Freshwater Sediments to Invertebrates

(U.S.) Corvallis Environmental Research Lab., OR

1984



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing

1. REPORT NO. EPA-600/J-84-184	2.	3. RE PB85-144103
4. TITLE AND SUBTITLE (Journal Version) Biological Methods for Determining Toxicity of Contaminated Freshwater Sediments to Invertebrates		5. REPORT DATE 1984 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Alan V. Nebeker, Michael A. Cairns, Jack H. Gakstatter Kenneth W. Malueg, Gerald S. Schuytema, Daniel Krawczyk		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Corvallis, Oregon 97333		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/02

15. SUPPLEMENTARY NOTES

Environmental Toxicology and Chemistry 3:617-630, 1984 (Oct-Dec)

16. ABSTRACT

Methods are presented for using Daphnia magna, Hyalella azteca, Gammarus lacustris, Chironomus tentans and Hexagenia limbata to screen freshwater sediments for acute and chronic toxicity, bioaccumulation potential and in situ toxicity. The 48-h Daphnia tests are recommended as inexpensive, uncomplicated and sensitive acute methods. Hyalella and Chironomus are the recommended test organisms as they are easy to rear and test, they remain in intimate contact with the sediment and they exhibit high control survival. Verification studies (published elsewhere) evaluating the recommended methods and organisms are briefly summarized.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	Unclassified	15
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/J-84-184
JOURNAL ARTICLE

Environmental Toxicology and Chemistry, Vol. 3, pp. 617–630, 1984
Printed in the USA. Pergamon Press Ltd.

0730-7268/84 $3.00 + .00
1984 SETAC

BIOLOGICAL METHODS FOR DETERMINING TOXICITY OF CONTAMINATED FRESHWATER SEDIMENTS TO INVERTEBRATES

ALAN V. NEBEKER,* MICHAEL A. CAIRNS, JACK H. GAKSTATTER, KENNETH W. MALUEG, GERALD S. SCHUYTEMA and DANIEL F. KRAWCZYK

U.S. Environmental Protection Agency
Corvallis Environmental Research Laboratory
Corvallis, Oregon 97333

(*Received* 4 *November* 1983; *Accepted* 14 *February* 1984)

Abstract — Methods are presented for using *Daphnia magna*, *Hyalella azteca*, *Gammarus lacustris*, *Chironomus tentans* and *Hexagenia limbata* to screen freshwater sediments for acute and chronic toxicity, bioaccumulation potential and *in situ* toxicity. The 48-h *Daphnia* tests are recommended as inexpensive, uncomplicated and sensitive acute methods. *Hyalella* and *Chironomus* are the recommended benthic test organisms, as they are easy to rear and test, they remain in intimate contact with the sediment and they exhibit high control survival. Verification studies (published elsewhere) evaluating the recommended methods and organisms are briefly summarized.

Keywords — Sediment bioassay methods Acute toxicity tests Chronic toxicity tests
Freshwater invertebrates

INTRODUCTION

This article describes rearing procedures, acute and chronic testing methods and bioaccumulation and *in situ* methods for estimating the potential impact of contaminated sediments on aquatic life. The specific tests presented here to screen freshwater sediments for toxicity use five freshwater invertebrates: *Daphnia magna* (cladoceran), *Hyalella azteca* (amphipod), *Chironomus tentans* (midge), *Gammarus lacustris* (amphipod) and *Hexagenia limbata* (mayfly).

Three types of acute tests are described: (a) a liquid phase elutriate test using *Daphnia*; (b) a solid phase sediment and water beaker test using one or several of the five organisms; and (c) a sediment and water Prater-Anderson type test using *Daphnia* and *Hexagenia*.

Four chronic tests are described: (a) a larval survival and growth test with *Chironomus*; (b) an adult-emergence test with *Chironomus*; (c) a *Hyalella* partial life cycle test; and (d) a *Daphnia* life cycle test.

Laboratory bioconcentration test methods are described, and an *in situ* test is discussed that may be used in the field for toxicity studies, laboratory–field comparisons or field bioconcentration determinations. A brief summary of verification tests (published elsewhere) conducted to evaluate the proposed methods is presented, along with a literature review and criteria to be used for test selection.

*To whom correspondence may be addressed.
Mention of trade names or commerical products does not constitute endorsement or recommendation for use.

Testing procedures approximate worst-case conditions, as potential toxic materials from the sediment will be retained in the water in the beakers of both the elutriate test, containing only dissolved materials, and the tests containing water and solid phase sediment. In the solid phase sediment and water tests, toxicants bound to particulates may also be available to the animals when they feed on or come in contact with the sediment.

The acute procedures recommended here are relatively inexpensive first-step screening processes to determine short-term acute toxic effects of contaminated sediments; they cannot indicate whether or not the sediments are free of toxic effects. Longer-term exposure, chronic life cycle tests, bioconcentration tests, *in situ* assays and assessment of invertebrate populations in the field are needed to show that the sediments are a hazard to freshwater aquatic life.

METHODS

Rearing of animals

All culture and testing procedures are done using a photoperiod of 16 h light:8 h dark, with all temperatures at 20°C, except for *Gammarus*, which are reared and tested at 16°C.

D. magna. *Daphnia* are reared in 4-liter jars containing 3 liters of water maintained at a hardness of >100 mg/L as $CaCO_3$ (a lower hardness reduced survival and young production; 200 mg/L is recommended). Only 50 adults are contained in each jar, to ensure adequate oxygen concentration without aeration. Eight jars are maintained in the culture, with two jars containing new age groups added each week, discarding those >30 d old. The water is changed two or three times each week, with some food being added daily. The water is poured through a 1.5-mm mesh screen (10 × 15 cm) to retain adults, and they are transferred to a jar of fresh water and new food added. The young to be used for testing or starting new cultures are retained by a fine screen (<0.5-mm mesh, 11 × 16 cm, with upturned edges) placed below the 1.5-mm mesh screen. At each fresh water change *Daphnia* are fed algae (*Selenastrum capricornutum*) and a fish food (Oregon Moist Pellet®) and yeast mixture [1] at a dry solids concentration of 2.0 mg/L algae and 5 mg/L fish food–yeast. On days when water is not changed they are fed 0.5 mg/L algae.

H. azteca *and* G. lacustris. *Hyalella* are the preferred species as they reproduce continually and grow rapidly, and burrow in the sediment when disturbed. *Hyalella* and *Gammarus* should be reared in 10- or 20-liter aquaria under flowing-water conditions if possible. *Gammarus* require several weeks of a short light period to initiate reproduction (e.g., 10 h light:14 h dark at 10°C). If static conditions are used, the water should be partially (e.g., 30%) changed once a week and gently aerated. It is unnecessary to maintain carefully controlled feeding regimens for *Hyalella* and *Gammarus*, as is done for *Daphnia*. Dried maple, alder, birch or poplar leaves (presoaked for several days) are used as the primary substrate and food for *Hyalella* and *Gammarus*. Rabbit food pellets, Cerophyl®, fish food such as Tetra® Conditioning Food, frozen or newly hatched brine shrimp or heat-killed young *Daphnia* serve as good food if used sparingly. To clean the rearing chamber or reduce populations of animals when they become abundant, transfer half of the leaf substrate (containing some animals) to a sorting tray, discard the remainder of the old chamber contents and return the leaf substrate and animals to the chamber. *Hyalella* will need to be thinned periodically because the population expands rapidly.

C. tentans. Midge cultures in flow-through 10-liter aquaria have been successful [2,3], but static test containers can be used if they are aerated and the water is

changed partially each week. Two egg masses are placed in an aquarium covered with an adult flight cage [3], with water flowing through the aquarium (50–100 ml/min) if possible. When eggs hatch (at 3 d), and once again 3 d later, 100 mg (0.25 ml dry volume) Cerophyl is placed in the aquaria for food and substrate (the larvae bind the Cerophyl together with silk to form tubes in which they live). The Cerophyl is mixed with water and then poured into the rearing chamber; several aquaria can be fed from a slurry prepared in one beaker. Finely powdered Tetra (10% of Cerophyl volume) can be added for a more balanced diet, especially in static containers where there is no nutrient input from the flowing water source. Two hundred milligrams (0.5 ml dry volume) Cerophyl (plus 10% Tetra) is added to the container twice a week thereafter; more may be needed with older larvae. Too much food will cause a fungal mat to develop that will kill young midges if it is not broken up; too little food will cause the midges to scrape bare spots on the container bottom between larval tubes. They will leave their burrows if insufficient food is available, so maintain a light "dusting" of fresh Cerophyl on the bottom of the container. The buildup of undigestible material over the 30 d of the life cycle provides the large mass of tube-building material required by the third- and fourth-instar larvae.

Second-instar larvae for use in testing can be collected from the aquarium 10 d after egg hatch (at 20°C). If several containers are set up [3] and eggs are added to each new container at 3-d intervals, larvae for tests will always be available and adults and eggs will be available for starting new cultures.

H. limbata. This species requires a substrate, but unlike the midges, they do not construct it from particles such as Cerophyl. They require a fine-textured high-organic sediment into which they can burrow and which will retain the burrow integrity (e.g., sand will collapse). The ideal substrate would be from the animal's native area. If eggs or young animals are received from a vendor (e.g., bait dealer or research laboratory), then clean sediment (free of predators) should be collected and used as substrate. It is difficult to determine how much to feed the larvae, especially when different-sized animals are in the culture. However, the addition of 1% by volume of an 80:20 mix of Cerophyl and Tetra to the chamber [e.g., 20 ml (8 g) food to 2,000 ml sediment in a 20-liter aquarium] will provide sufficient food to initiate the culture. Weekly additions of the same amount should maintain the culture, unless fungus appears on the sediment surface, which indicates that too much food is being added.

A flow-through rearing facility [3] should be used, because the water will remain clear and the animals and burrows (or fungus if overfed) can be easily observed. If static tanks are used (with concomitant turbid water of near-zero visibility), gentle aeration and partial (e.g., 20%) weekly water change are required. Five centimeters of substrate is adequate for culture of *Hexagenia*. Brushing or stirring the sediment surface is useful for circulating the food across the mud surface and for determining numbers of animals in the holding tank in flow-through systems, because the larvae will rapidly form new burrows, which facilitates counting. Five hundred newly hatched or 100 older larvae (up to 15 mm in length) can easily be maintained in a 40-liter aquarium.

Acute test methods

Three acute test methods are described: (a) the liquid phase elutriate test in which water is mixed with contaminated sediment (1:4, v/v, mix of sediment and water) and then settled and centrifuged—the water is used to test *Daphnia* by exposing them to dissolved materials extracted from the sediment; (b) the solid phase sediment and water test in which sediment and water (1:4, v/v, ratio of sediment and water), together in the test chamber, are used to expose the test species to both dissolved and bound materials in the sediments; and (c) the solid phase

sediment and water Prater-Anderson test (1:9.5, v/v) in which *Hexagenia* are placed in the sediment and *Daphnia* suspended in a cage in the recirculating water column.

Appropriate statistical analyses to determine significant differences in animal survival between test sediments and control sediments are required. Chi-square analysis can be used to test for differences between control and test survival at any desired level of significance.

Routine chemical analyses such as dissolved oxygen, pH, total hardness and total alkalinity should be conducted to assure that the water is satisfactory for the animals. Other analyses such as particle size, organic content, heavy metals, ammonia, organics, etc., may be required to better characterize the sediment.

Sediment samples are collected and held at 4°C until used (preferably <2 weeks of storage). They should be thoroughly mixed and screened through one or more standard sieve screens to remove large particles and endemic animals, especially predators. An uncontaminated control sediment with similar particle size and organic content should be collected and treated similarly.

To obtain <24-h-old *Daphnia* for testing, adults and young in rearing jars (screened the day before to remove young) are poured onto a 10 cm × 15 cm screen of 1.5-mm mesh to retain adults only, which are returned to the rearing jars. A 11 cm × 16 cm fine screen (<0.5 mm mesh) with upturned edges is placed under the 1.5-mm mesh screen to retain young, which are then transferred to other containers. *Daphnia* are counted and transferred to test containers with a 5 mm inner diameter glass pipette. For solid phase testing, young are fed and reared for 5 d before testing so that they will be large enough to recover at the termination of the test.

Transferring the other animals from rearing containers to test beakers is relatively easy. *Hyalella* and *Gammarus* are separated from the leaf material in their rearing chamber by scooping up the leaves (containing animals) and placing them on a 5 to 10-mm mesh plastic screen placed over a white porcelain pan containing 2 cm water. By sprinkling water on the leaves while turning and separating them, the animals are washed from the leaves and drop through the screen into the pan and are immediately available for use. *Hexagenia* can be screened and rinsed from the rearing substrate with a 2-mm mesh screen and rinsed into the pan. The midges can be pipetted directly from the water containing them and their substrate (poured from the rearing chamber into the pan) because they swim free of their burrows when disturbed by water turbulence. *Hyalella, Chironomus* and young *Gammarus* can be transferred from the pan to test chambers with a 7 mm inner diameter glass pipette. *Hexagenia* and larger *Gammarus* may be transferred with a spoon-shaped piece of screen.

Liquid phase elutriate test with D. magna. This procedure is adapted from the methods described in the joint U.S. Environmental Protection Agency (EPA)–U.S. Army Corps of Engineers manual [4] for preparation of liquid phase marine dredge samples. *Daphnia* are exposed for 48 h to centrifuged water samples obtained from a sediment–water slurry. The sediment is mixed with clean dilution water in a volumetric sediment-to-water ratio of 1:4 and placed in a closed container (e.g., 350 ml sediment/1,400 ml water in 2-liter bottle) and mixed vigorously for 30 min. The samples are then allowed to settle overnight. The overlying water is siphoned off and centrifuged at 10,000 rpm for 15 min. (Centrifugation has been shown to be more efficient than 0.45-μm filtering in removing undissolved components from the water [W. L. Griffis, U.S. EPA, Corvallis, OR, personal communication].) The water (200 ml/beaker) is then transferred directly to three 250-ml beakers. They are gently aerated with glass-tipped plastic air lines, with the tip 1 cm below the water surface.

Ten *Daphnia* (< 24 h old) are placed in each of the three beakers containing the elutriate water to be tested. If the sample kills all animals in 48 h, the centrifugate can be diluted or a new sample can be prepared and diluted several times to determine relative toxicity of the sediment sample.

Solid phase sediment and water beaker test. These tests, conducted in aerated 1,000-ml beakers [3], expose *Daphnia* for 48 h and *Hyalella, Chironomus, Gammarus* and *Hexagenia* for 10 d, at which time survivors are screened from the water and sediment and counted, using survival as the criterion for toxicity.

Sediment (200 ml) is placed in each of three replicate 1,000-ml glass beakers. After addition of the sediment, 800 ml of dilution water is gently poured into each beaker, bringing the total contents to 1,000 ml. Beakers should be left unaerated overnight to reduce turbidity and to allow more time for water–sediment contact before animals are placed in the beakers. The water in the beakers should be aerated for 30 min before test animals are added, using glass-tipped plastic air lines from an air source. Gentle aeration with the tip 3 cm below the water surface is used to aerate the water, avoiding any disturbance of the sediment that would create unnecessary turbidity.

Fifteen test animals are then placed in each beaker: Of those animals studied, 5-d-old *Daphnia*, juvenile *Hyalella* and 2nd-instar *Chironomus* larvae were found to be the easiest to rear and test in the laboratory. *Daphnia* and *Chironomus* can be tested together in the same beaker. Ten early-instar *Hexagenia* (< 10 mm long) or juvenile *Gammarus* (< 7 mm long) may also be used in each beaker. Cannibalism and high control mortality results from using larger *Gammarus* and *Hexagenia* in the 1,000-ml beakers. However, larger containers with more sediment, such as 4-liter jars, can be used (10 animals/jar) if small animals are not available. At the end of the test the sediment is screened (0.5–2-mm mesh depending on animal size) to collect and count the live and dead animals remaining in the sediment. Good *Daphnia* recovery after 48 h is obtained by pouring water and fine suspended sediment, but not the bulk of the sediment, through the 10 cm × 15 cm (0.5-mm mesh) screen. If *Daphnia* and *Chironomus* are tested together, the water and fine sediment can be returned to the beaker with *Chironomus* for completion of the 10-d exposure. The *Daphnia* are then gently rinsed from the screen and transferred to clean water for counting.

Solid phase sediment and water Prater-Anderson test. Use of this system [5,6] containing both *Daphnia* and *Hexagenia* (20 and 5 per replicate, respectively) will generally give results comparable to those of the beaker test [7]. However, this test is more time-consuming to construct, calibrate and use for bioassays, so the beaker tests are preferable. In beaker tests, *Daphnia* have direct contact with the sediment, whereas with the Prater-Anderson device, they are retained in a cage in the water column. The *Daphnia* (< 24 hr old) in the Prater-Anderson system are exposed to dissolved and particulate materials due to *Hexagenia* activity. The Prater-Anderson system does have larger volumes of sediment and water, as well as a larger water/sediment ratio (9.5:1), so that larger *Hexagenia* can be used and more water samples can be removed to monitor chemical conditions.

Chronic tests

Chironomus *adult emergence test.* This 25-d test is adapted from the midge life cycle test found in *Standard Methods* [8], and is a relatively inexpensive procedure designed to encompass most of the life cycle of this animal. The test is started with 10-d-old (20°C) second-instar larvae, and the endpoint is a count of emerged adults.

The sediment to be tested is placed in containers, such as 20-liter aquaria or 4-liter jars, and covered by screening to retain adults. The sediment layer should be 2 to 3

cm deep, overlaid with 15 cm of gently aerated water. Distilled water is used to replace water lost by evaporation. One hundred larvae are added to the sediment at the start of the test. A food mixture of 600 mg Cerophyl (1.5 ml dry volume) and 100 mg (0.3 ml) finely crushed Tetra flakes should be mixed with distilled water in a small beaker and fed to the animals at the start of the test and again on day 8. On day 14 they should be fed 800 mg (2.0 ml) Cerophyl and 100 mg (0.3 ml) Tetra, and on day 18 they should be fed 1,000 mg (2.5 ml) Cerophyl and 100 mg (0.3 ml) Tetra. If larvae are dying and not consuming the food, fungus will appear, indicating that less food should be added. Adults should begin emerging after 20 d; the test should be continued for another 5 d to count all adults emerging and to observe for delayed development. A small vacuum pump with a 10-mm diameter plastic line running through an Erlenmeyer flask trap is used to collect adults and make daily counts of those emerging (the screen cover is slowly and gently lifted off the container, and the adults are vacuumed from the screen and the inside walls of the container).

Chironomus *larval survival and growth test.* If the larvae need to be retained for bioconcentration studies, follow the same initial procedures used for the adult emergence test, but use larval survival and growth (length and weight) after 15 d as the endpoints of the chronic test. The sediment can be screened to collect the larvae, which are placed in water only overnight to clear the gut. They are then killed with warm water, blotted dry, weighed (mg) and measured (mm) and frozen for later tissue analysis.

D. magna *life cycle test.* This relatively inexpensive test is started with 5-d-old *Daphnia* and exposes them for 10 d, through maturation and release of young (three broods), at which time the test is terminated and adults and young are counted. Total number of surviving adults and young, compared to controls, is the criterion for establishing sediment toxicity.

Four-liter jars, holding 2.5 liters of water plus 500 ml of test sediment, can be used as test containers. The test should be started by adding 20 5-d-old *Daphnia* to each jar along with food at the rate of 2 mg/L solids (1.0 mg/L algae such as *Selenastrum*, plus 1 mg/L solids from the fish food–yeast mixture). They should be fed every other day until the end of the 10-d test. The containers should be gently aerated with a glass-tipped plastic air line, with the tip 4 cm below the water surface (sufficient to aerate the water without disturbing the sediment). At the end of the test, water and fine suspended solids, but not the bulk of the sediment, is poured through the 0.5-mm mesh (10 cm × 15 cm) screen to retain surviving adults and young *Daphnia*. They are then gently rinsed from the screen and transferred to clean water for counting.

Hyalella *partial life cycle test.* Use the methods described for the *Hyalella* bioconcentration test. Endpoints for the 28-d chronic exposure are the number of adults and young surviving. Sediment should be screened through a 1.0-mm mesh standard sieve before testing, if possible (may be diluted and mixed 1:1 with test water to facilitate screening), as particles should be smaller than the young animals so they can be effectively screened (0.5-mm mesh) and recovered from the sediment at the end of the test.

Bioconcentration tests

These methods are designed to expose *Hyalella, Gammarus* or *Hexagenia* for 28 d, and *Chironomus* for 15 d, after which the animals are removed for tissue analysis of the toxicant(s) being monitored.

The test containers are 20-liter aquaria with 2 to 3 cm of test sediment on the bottom overlaid with 15 cm water. If less sediment is available for testing, 4-liter jars can be used, but proportionally fewer animals and less food should be used. Sediment

should be screened so the particles are smaller than the animals to be tested, if possible, so that the animals can be more easily screened from the sediment at the end of the test.

The test, using 20-liter aquaria, is started with 100 *Hyalella* or juvenile *Gammarus* (<7 mm long), second-instar *Chironomus* or early-instar (10–15 mm) *Hexagenia*. If larger *Gammarus* or *Hexagenia* are used, 25 to 50 animals should be used. They should be placed in each of two or more replicate aquaria and exposed for 28 d (15 d for *Chironomus* larvae). At the end of the test, animals should be screened from the test water and sediment, placed in clean water overnight to clear the gut and then frozen for later analysis.

Food for the animals, in addition to what they obtain from the sediment sample, should be Cerophyl and Tetra for *Chironomus* and *Hexagenia*, and rabbit food pellets for *Hyalella* and *Gammarus*. The Cerophyl–Tetra food mixture [600 mg Cerophyl (1.5 ml dry volume) and 100 mg (0.3 ml) powdered Tetra] should be mixed with 100 ml distilled water in a small beaker and dispersed over the water surface. Give 200 mg (0.5 ml dry volume) rabbit food (soaked and dispersed in 100 ml distilled water) to *Hyalella* and 600 mg (1.5 ml) to *Gammarus* at each feeding. Animals should be fed twice a week during the exposure period. If mortalities occur, feeding should be reduced proportionally. All tanks should be aerated and water lost to evaporation should be replaced with distilled water.

In situ *test*

The basic concept of an *in situ* bioassay for benthic invertebrates is to expose the test animals at the field site, without disturbing the contaminated sediment, and determine percent survival. With fish tests for water-only exposure the process is simple: A cage is hung in the water column or anchored to the bottom and the fish are transferred to the cage and exposed for 96 h or longer. Testing invertebrates in the undisturbed sediment poses more problems, such as predation and recovery of the animals.

A 9 cm × 20 cm cylindrical stainless steel screen (1.5-mm mesh) cage is used to contain the test animals. The cage is closed with a petri dish at one end, with another petri dish placed over the open end after the test animals are introduced. Twenty adult *Hyalella*, juvenile (7–10 mm) *Gammarus*, early-instar (10–15 mm) *Hexagenia* or third-instar *Chironomus* should be used in each cage. Only 10 larger *Gammarus* or *Hexagenia* should be used, and all should be of the same length. If *Chironomus* are used, the screen should be 0.75-mm mesh rather than 1.5-mm, but this mesh can only be used with sediments of very small particle size. The animals are not fed during the exposure. An uncontaminated control site with similar sediment conditions should be selected to expose and recover control test animals. The animals are acclimated to site temperature, and are carried to the test site in a container with water. The cage is partially submersed in water at the test site, the animals gently added and the cover fitted securely. One-fourth of the cage, if possible, is then gently forced lengthwise into the sediment and supported with stakes. The animals are exposed for 96 h using at least two replicate cages. The cage is then removed from the sediment (with the cover still in place), gently washed free of sediment and the enclosed test animals removed and counted.

TEST METHODS EVALUATION STUDIES

A series of tests was conducted at this laboratory, using field-contaminated and artificially spiked sediments, to develop and validate the proposed methods. The results of these evaluation studies (published elsewhere) are briefly summarized here.

Malueg et al. [7] used the test apparatus developed by Prater and Anderson [5] with *Daphnia* and *Hexagenia* to determine the toxicity of several contaminated sediments, and to confirm the suitability of the two

species as test organisms for screening sediments for acute toxicity. They found *Daphnia* to be more sensitive to sediment toxicants than *Hexagenia*, and in some paired sediment tests with and without *Hexagenia*, *Daphnia* died only in test chambers containing *Hexagenia*, probably because the higher turbidity created by the burrowing mayfly caused greater release of toxicants.

Malueg et al. [9] conducted tests using the Prater-Anderson apparatus with *Daphnia* using sediment from three areas contaminated with copper and other heavy metals. They found a good correlation between laboratory tests and field observations of the harmful impacts on the invertebrates, indicating that laboratory tests may predict freshwater sediment toxicity from samples collected from suspected areas. They also conducted tests using sediment from the Keweenaw Waterway, MI [10], and showed direct sediment–copper toxicity relationships with *Daphnia* and *Hexagenia*, and with distribution of benthic macroinvertebrates.

Cairns et al. (manuscript in preparation) conducted a series of tests using *Daphnia* with the same sediments used by Malueg et al. [9] but with two types of tests—the liquid phase elutriate test in aerated 250-ml beakers, and the solid phase sediment and water test in aerated 1,000-ml beakers. Both types of tests produced good data, with good control survival and replicates and with results similar to those of Malueg et al.

Cairns et al. [11] and Nebeker et al. (manuscript in preparation) conducted elutriate and solid phase beaker tests with *Daphnia*, *Chironomus*, *Hyalella*, *Gammarus* and *Hexagenia* using field-contaminated sediments and clean sediments artificially spiked with copper and cadmium. *Daphnia* was the most sensitive animal tested (48-h tests) while the other animals were generally similar in their sensitivity (10-d exposures), with no one animal being consistently more or less sensitive. Control survival was satisfactory for *Daphnia*, *Chironomus* and *Hyalella* without addition of food. However, larger *Gammarus* and *Hexagenia* exhibited excessive control mortality in the beakers due to competition for space and cannibalism. Tests using the larger 4-liter jars, rather than the 1-liter beakers, were satisfactory for *Gammarus* and *Hexagenia*. Flow-through tests were also completed and compared to the static tests with cadmium-contaminated sediments (Nebeker et al., manuscript in preparation). Good survival of *Chironomus*, *Hyalella* and *Daphnia* occurred in flow-through tests, whereas significant mortality occurred in static tests, indicating that the animals were being killed by cadmium released into the water column and concentrated to higher levels in the static tests than in the flow-through tests.

A study by Schuytema et al. [12] with *Daphnia* using cadmium-spiked sediment slurries again demonstrated the usefulness of *Daphnia* as a test animal. Results of the research showed that, at least for cadmium and *D. magna*, free cadmium ion was causing most of the toxicity; cadmium bound to the particulates in suspension apparently was not available.

Tests conducted to determine the suitability of older *Daphnia* for sediment studies, because they are much easier to screen from turbid water and sediment (Nebeker et al., manuscript in preparation) showed that, in general, 5-d-old *Daphnia* had EC_{50} values similar to the < 24-h-old daphnids for copper, cadmium and cyanazine. The data suggest that the use of 5-d-old *Daphnia*, rather than < 24-h-old animals, will not result in differences in sensitivity. A study by Nebeker et al. [2] conducted with *Chironomus* determined that 10-d-old second-instar larvae were most suitable for starting tests. They are large enough to work with for reliable results, are the most sensitive of the three larger larval instars, and allow two larval molts (second to third and third to fourth instar) during a 10-d acute exposure. However, they should be replaced by third-instar larvae in *in situ* tests, as third instar

larvae are larger, allowing use of larger-mesh screen for cages.

A study completed by Knight [13] sought to determine suitable freshwater aquatic invertebrates for use in sediment screening studies and to develop and validate methods using those animals selected. The freshwater clam *Corbicula fluminea* was found to be very useful for long-term field monitoring and bioaccumulation studies, and is now being employed in the Sacramento River delta area for that purpose. However, it was not found to be useful in acute studies because it often closes its shell when exposed to toxic materials. Several other candidate invertebrates were screened and the midge *Chironomus decorus* and the freshwater amphipod *Corophium stimpsoni* were chosen for further study. *Corophium* was found to do well in Sacramento River water but the addition of some chloride was needed in laboratory studies. Rearing, life cycle and toxicity studies with heavy metals were completed with the midge *Chironomus*, and it was recommended as a useful animal for screening potentially toxic sediments.

Tests were conducted to validate the usefulness of the *Chironomus*, *Daphnia* and *Hyalella* chronic tests, and the bioconcentration tests (Nebeker et al., manuscript in preparation). Three adult emergence tests with second-instar *Chironomus* were sucessfully completed, with 45 to 70% adult emergence from control and toxic sediments. One test starting with third-instar larvae averaged 65% emergence (range 56–74%); results were similar between 20-liter aquaria and 4-liter jars. Five *Daphnia* chronic tests (10-d) were completed successfully. Counting total number of adults and young after 10 d gave an average of 97% survival of adult *Daphnia* in the controls, and an average of 408 young were recovered from each test jar. Several bioconcentration tests and partial life cycle tests with *Hyalella* were completed, with recovery of adult animals ranging from 21 to 95%. However, it was difficult to recover young due to their small size, especially when sediment was not screened prior to testing. *Chironomus* bioconcentration and larval survival tests were completed successfully, with larval recoveries ranging from 68 to 91%.

Nebeker and others at the Corvallis EPA laboratory are presently conducting tests with the annelid worm *Lumbriculus variegatus* to determine its usefulness as a sediment test organism for acute, chronic and bioconcentration tests. Studies to date show that the worms have a sensitivity similar to that of *Daphnia* in copper mine–effluent sediment, but are more tolerant of sediment from harbors containing mixed contaminants.

DISCUSSION AND LITERATURE REVIEW

Several excellent articles on culture, rearing and testing of the various test animals are available. *Standard Methods* [8] presents information on collecting and holding test animals and on testing procedures. The American Society for Testing and Materials [1,14] and Buikema et al. [15] give detailed procedures for culturing and rearing *D. magna*. Arthur [16] reviews bioassay procedures for amphipods with specific reference to *Gammarus*. Culture methods for *D. magna*, *H. azteca*, *G. lacustris*, *C. tentans* and *H. rigida* can be found in the *Manual for Culture of Selected Freshwater Invertebrates* [17]. Fremling and Mauck [18] also present methods for using nymphs of burrowing mayflies as toxicity test organisms.

Acute tests completed at this laboratory with freshwater invertebrates were conducted using some methods similar to those used successfully by others, such as the modified freshwater Prater-Anderson system [5,6], and the 1,000-ml beaker method used by Swartz et al. [19] for marine benthic invertebrates. The elutriate test using *D. magna* in aerated 250-ml beakers with water containing only dissolved materials is adapted from the procedures recommended by U.S. EPA–U.S. Army Corps of Engineers

[4], and has been validated by several researchers [20–23] as a successful tool for screening potentially toxic sediments.

A report by Plumb [23] on procedures for handling and chemical analysis of sediment and water samples provides state-of-the-art guidance on sampling, preservation and analysis of sediments. It is a summary of methods currently in use and should be consulted for detailed procedures for handling sediments up to when they are used in bioassays. The review does not give bioassay methods, but does briefly summarize the elutriate procedure and gives excellent references for all phases of sediment collection, handling and chemical analyses.

Chapman et al. [24] present a brief summary in a convenient tabular form of the current knowledge regarding the fate and effects of priority pollutants. They list persistence, accumulative capacity and volatility, and the environmental compartment (water, sediment or biota) in which pollutants will most likely be found. They emphasize that the overall task of developing both a comprehensive and cost-effective environmental monitoring program for priority pollutants can be greatly simplified and improved by first knowing the relative importance of each pollutant and the environmental compartment of major concern.

Adams et al. [25] used the midge *C. tentans* to define the key route of exposure (interstitial water, water column water, sediment or food) for kepone in partial life cycle static and flow-through tests, using survival, growth and bioaccumulation as endpoints. They used 3-liter aquaria containing 100 g dry sediment that had been screened through a No. 25 (710-μm opening) standard sieve to remove large particles. They dosed the sediment with kepone using dry sediment to which acetone containing the desired amount of kepone was added. A slurry of 75 ml acetone (containing kepone) and 100 g soil was stirred and placed under a hood to evaporate the acetone. Water was then added to the sediment and 25 midge larvae were placed in the aquaria. They concluded that interstitial water was the primary route of exposure. Their results were excellent, and their sediment-spiking method using organics should work well as a standard method.

Seelye and Mac [26] present a literature review on the effects of dredging activities on freshwater organisms, and recommend the following: (a) Static tests should be avoided due to problems with low dissolved oxygen. (b) The elutriate test may not be of value. (c) Tests on dissolved or suspended particulate phases may not be of value. (d) Bioaccumulation studies should be conducted and the exposures should last at least 10 d, preferably > 30 d. (e) Whole sediment (solid phase) tests should be conducted and toxicity and bioaccumulation tests should be combined to reduce costs. (f) Sediment characteristics at the site should be defined because they greatly affect toxicant adsorption and availability. (g) Sublethal parameters such as reproductive success, growth abnormalities and avoidance should be considered for testing. (h) Use of flow-through tests should be encouraged. (i) Test animals should come in contact with the sediments. (j) Test 1 (elutriate) and test 2 (suspended particulates) of the U.S. EPA–U.S. Army Corps of Engineers methods manual [4] should not be used—only test 3, the whole sediment (solid phase) test, should be used.

We found that static tests were very useful and that aeration of the test containers eliminated the oxygen problem [11]. The elutriate test was a valuable addition to the solid phase test, because the combination showed when the sediment was toxic but was not releasing dissolved materials into the overlying water column: One of the toxic sediments from Wisconsin, with 27% organic content, was not toxic in the elutriate test but was toxic when animals had access to the solid phase sediment. Most of the sediments that caused mortality in test animals showed toxicity in both the elutriate and the solid phase tests, usually due to

high dissolved concentrations that leached into the water (desorbed) from the sediment.

The question of freezing sediments versus storing them at 4°C until testing still needs resolution. A. Gahler (U.S. EPA, Region 10, Seattle, WA, personal communication) reported that freezing and defrosting alters release of nitrogen and phosphorus from sediments. J. Cummins (EPA Region 10, personal communication) observed that when frozen sediments are used toxicity is reduced by 20 to 30%. T. Dillon (poster presentation, SETAC Meeting, Arlington, VA, November 6–9, 1983) conducted studies on the effects of time and storage temperature (−22, 4 and 25°C) on sediment toxicity and recommended that sediment be stored at 4°C and used within 2 weeks if possible. We are presently conducting freezing (−20°C) versus 5°C storage tests to determine impacts on sediment chemistry and toxicity. Results to date show that freezing significantly reduces the toxicity to *Daphnia* of sediment spiked with copper. Caution is recommended in freezing sediment unless data are available to show what changes occur.

Bahnick et al. [20] conducted extensive acute 96-h tests and bioconcentration studies with *Hexagenia*, the amphipod *Pontoporeia* (native to the Great Lakes) and *Daphnia* using sediments from the Duluth-Superior Harbor and Lake Superior. Their procedures used sediment with overlying water (solid phase), tests with interstitial water and tests using elutriate water. The results demonstrated that the animals could be maintained successfully in complex test systems with good survival in controls, and in most of the sediments tested. *D. magna* was the most sensitive to the contaminated sediments.

Prater and Anderson [5,6] and Hoke and Prater [27] used a unique recirculating bioassay chamber and conducted an extensive series of bioassays to develop methodology and generate data. They used *Daphnia*, *Hexagenia* and other invertebrates, and screened many sediments from the Upper Midwest and Great Lakes areas. Their methods were verified by Malueg et al. [7] as being useful in characterizing the toxicity of heavily contaminated sediments. Further attempts were made to sort out cause and effect relationships between chemical contaminants and biological responses [27,28] but without great success. They concluded that bulk chemistry correlated better with test species mortality than did elutriate (soluble) chemical measurements; however, that conclusion was disputed by Lee and Jones [29], who support the elutriate chemical test.

In laboratory tests, significant effects of contaminated sediment from Palestine Lake, IN, were demonstrated on the survival and growth of larvae of the midge *C. tentans* [30], and emergence of midge adults was reduced to one-third of control levels [31]. Shuba et al. [32] conducted several flow-through bioassays on kepone-contaminated sediments from Bailey Creek and the James River in Virginia, and also on sediments from a small stream receiving the Vicksburg, MS, sewage treatment plant effluent. Because their primary interest was in developing tests to predict effects of dredge-spoil disposal, they used a variety of sediment treatments, exposure times and test organisms (*Daphnia, Corbicula, Palaemonetes, Musculium* and *Lirceus*). They recommended *Paleomonetes, Daphnia* and *Musculium* as test species for acute toxicity studies and *Corbicula* for bioaccumulation studies. In our studies, we found *Daphnia* to be most sensitive, and Knight et al. [13] recommended *Corbicula* for bioaccumulation studies.

Marking et al. [33] conducted static toxicity tests with sediments from 10 sites on the upper Mississippi River, using *Gammarus, Procambarus, Chironomus, Physa, Truncilla* and *Sphaerium*. Sediments for 2 of the 10 sites were toxic to one or more test species, but no consistent responses of the test species were observed. Gannon and Beeton [34] conducted bioassays on sediments from nine Great Lakes harbors using the am-

phipod *Pontoporeia affinis*, an important component of the Great Lakes benthic community. About one-third of the sediments tested were toxic (50% mortality) to the test organism. Bailey and Liu [35] demonstrated the potential of the freshwater oligochaete *Lumbriculus variegatus* as a bioassay organism in water only and gave methods for rearing and testing. It has potential importance in toxicity and bioconcentration studies with contaminated sediments because it maintains intimate contact with the sediment.

Nearly all of the sediment toxicity investigations reported here used static exposure systems rather than flow-through tests, which seems appropriate as a conservative approach for predictive or assessment purposes. Evidence to date indicates that, generally, biologically available sediment contaminants are those released back into the water. A static exposure system allows the contaminants to accumulate and affect the test organism in a worst-case type of environment, and the potential toxicity of a sediment can be determined.

TEST SELECTION CRITERIA

Proper selection of test method or test animal is necessary to obtain the most useful information, especially with limited resources.

The choice of the bioconcentration test is obvious when there is a need to determine tissue levels of a chemical known to occur in the sediment under investigation. The decision as to which acute or chronic toxicity test to use may be more difficult.

The 48-h solid phase (sediment and water) beaker test with *Daphnia*, in conjunction with the elutriate test, is recommended as a relatively fast, simple and inexpensive approach to initial screening for acute toxicity. Elutriate and solid phase tests conducted together are valuable because the combination shows when the sediment is toxic but is not releasing dissolved materials into the overlying water column. *Daphnia* generally have been shown to be the most sensitive animals used for sediment tests, especially to metals, and are the logical first choice. They are easy to rear and handle in the laboratory and there is a large database on *Daphnia* available from freshwater tests. The tests are relatively inexpensive, require little special equipment and are of short (48-h) duration. Even though they are planktonic, *Daphnia* do feed at the surface of the sediments and come in contact with particulate-bound toxicants.

Hyalella and *Chironomus* are the recommended benthic organisms for use in solid phase tests, as they are easy to rear and test in the laboratory. They maintain intimate contact with and burrow in the sediment, consistently exhibit high control survival and are very sensitive to toxic organic chemicals. If the sediment has a high organic content, a test with *Hyalella* or *Chironomus* in addition to one with *Daphnia* would be appropriate to determine toxicity of the solid phase sediment rather than soluble toxicants only. *Daphnia* and *Chironomus* can be tested together in the same beaker. *Gammarus* and *Hexagenia* are more useful in tests combining toxicity and bioconcentration, in larger containers, because of the greater biomass available for tissue analysis.

Because of greater organism diversity, use of all five test organisms at the same time (in separate containers, except for *Daphnia* and *Chironomus*) is a valuable way to characterize sediment toxicity. With two replicate containers per organism, only 16 beakers would be required, using one contaminated sediment sample and a control.

The chronic tests have a valuable function in determining if impairment of growth and reproduction may occur in sediments that do not exhibit acute toxicity, portending disruption of biological integrity and the subsequent loss of invertebrate populations from impacted freshwater areas.

REFERENCES

1. **American Society for Testing and Materials**. 1982. Standard practice for conducting renewal life-cycle toxicity tests with *Daphnia magna* (Draft 1, Aug. Rita Comotto, E47.01).
2. **Nebeker, A.V., M.A. Cairns** and **C.M. Wise**. 1984. Relative sensitivity of *Chironomus tentans* life stages to copper. *Environ. Toxicol. Chem.* 3:151–158.
3. **Nebeker, A.V., P. McKinney** and **M.A. Cairns**. 1983. Acute and chronic effects of diflubenzuron (Dimilin) on freshwater fish and invertebrates. *Environ. Toxicol. Chem.* 2:329–336.
4. **U.S. Environmental Protection Agency/U.S. Army Corps of Engineers**. 1977. Ecological evaluation of proposed discharge of dredged material into ocean waters. Environmental Effects Laboratory, U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.
5. **Prater, B.L.** and **M.A. Anderson**. 1977. A 96-hour bioassay of Otter Creek, Ohio. *J. Water Pollut. Control Fed.* 49:2099–2106.
6. **Prater, B.L.** and **M.A. Anderson**. 1977. A 96-hour bioassay of Duluth and Superior harbor basins (Minnesota) using *Hexagenia limbata, Asellus communis, Daphnia magna,* and *Pimephales promelas* as test organisms. *Bull. Environ. Contam. Toxicol.* 18:159–168.
7. **Malueg, K.W., G.S. Schuytema, J.H. Gakstatter** and **D.F. Krawczyk**. 1983. Effect of *Hexagenia* on *Daphnia* response in sediment toxicity tests. *Environ. Toxicol. Chem.* 2:73–82.
8. **American Public Health Association/American Water Works Association/Water Pollution Control Federation**. 1980. *Standard Methods for the Examination of Water and Wastewater*, 15th ed. APHA, Washington, DC, pp 615–714.
9. **Malueg, K.W., G.S. Schuytema, J.H. Gakstatter** and **D.F. Krawczyk**. 1984. Toxicity of sediments from three metal-contaminated areas. *Environ. Toxicol. Chem.* 3:279–291.
10. **Malueg, K.W., G.S. Schuytema, D.F. Krawczyk** and **J.H. Gakstatter**. 1984. Laboratory sediment toxicity tests, sediment chemistry and distribution of benthic macroinvertebrates in sediments from the Keweenaw Waterway, Michigan. *Environ. Toxicol. Chem.* 3:233–242.
11. **Cairns, M.A., A.V. Nebeker, J.H. Gakstatter** and **W. Griffis**. 1984. Toxicity of copper-spiked sediments to freshwater invertebrates. *Environ. Toxicol. Chem.* 3:435–446.
12. **Schuytema, G.S., P.O. Nelson, K.W. Malueg, A. V. Nebeker, D.F. Krawczyk, A.K. Ratcliff** and **J. H. Gakstatter**. 1984. Toxicity of cadmium in water and sediment slurries to *Daphnia magna*. *Environ. Toxicol. Chem.* 3:293–308.
13. **Knight, A.W.** 1984. The evaluation of contaminated sediments employing selected benthic freshwater invertebrates. Final Report of the U.S. EPA Cooperative Agreement No. CR-808424. University of California, Davis, CA. Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR.
14. **American Society for Testing and Materials**. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E 729-80. American Society for Testing and Materials, Philadelphia, PA.
15. **Buikema, A.L., Jr., J.G. Geiger** and **D.R. Lee**. 1980. Daphnia toxicity tests. In A.L. Buikema, Jr., and John Cairns, Jr., eds., *Aquatic Invertebrate Bioassays*. ASTM STP 715. American Society for Testing and Materials, Philadelphia, PA, pp. 48–69.
16. **Arthur, J.W.** 1980. Review of freshwater bioassay procedures for selected amphipods. In A.L. Buikema, Jr., and John Cairns, Jr., eds., *Aquatic Invertebrate Bioassays*. ASTM STP 715. ASTM, Philadelphia, PA, pp. 98–108.
17. **Lawrence, S.G.**, ed. 1981. *Manual for the Culture of Selected Freshwater Invertebrates*. Can. Spec. Publ. Fish. Aquat. Sci. 54:169 p.
18. **Fremling, C.R.** and **W.L. Mauck**. 1980. Methods for using nymphs of burrowing mayflies (Ephemeroptera, *Hexagenia*) as toxicity test organisms. In A.L. Buikema, Jr., and John Cairns, Jr., eds., *Aquatic Invertebrate Bioassays*. ASTM STP 715. ASTM, Philadelphia, PA, pp. 81–97.
19. **Swartz, R.C., W.A. DeBen, K.A. Sercu** and **J.O. Lamberson**. 1982. Sediment toxicity and the distribution of amphipods in Commencement Bay, Washington, USA. *Mar. Pollut. Bull.* 13:359–364.
20. **Bahnick, D.A., W.A. Swenson, T.P. Markee, D.J. Call, C.A. Anderson** and **R.T. Morris**. 1980. Development of bioassay procedures for defining pollution of harbor sediments. Final Report of the U.S. EPA Project No. R804918-01. Center for Lake Superior Environmental Studies, University of Wisconsin, Superior, WI. Also: Project Summary. EPA-600/S3-81-025. June 1981. Environmental Research Laboratory, Duluth, MN.
21. **Lee, G.F., R.A. Jones, F.Y. Saleh, G.M. Mariani, D.H. Homer, J.S. Butler** and **P. Bandyopadhyay**. 1978. Evaluation of the elutriate test as a method of predicting contaminant release during open water disposal of dredged sediment and environmental impact of open water dredged material disposal, Volume II: Data report. Technical Report D78-45. U.S. Army Corps of Engineers, WES, Vicksburg, MS.
22. **Jones, R.A.** and **G.F. Lee**. 1978. Evaluation of the elutriate test as a method of predicting contaminant release during open water disposal of dredged sediment and environmental impact of open water dredged material disposal, Volume I: Discussion. Technical Report D78-45. U.S. Army Corps of Engineers, WES, Vicksburg, MS.
23. **Plumb, R.H., Jr.** 1981. Procedures for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE-81-1. U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.
24. **Chapman, P.M., G.P. Romberg** and **G.A. Vigers**. 1982. Design of monitoring studies for priority pollutants. *J. Water Pollut. Control Fed.* 54:292–297.
25. **Adams, W.J., R.A. Kimerle** and **R.G. Mosher**. 1983. Aquatic safety assessment of chemicals

sorbed to sediments. Presented at American Society for Testing and Materials 7th Annual Aquatic Toxicology Symposium, April 17–19, 1983, Milwaukee, WI.

26. **Seelye, J.G.** and **M.J. Mac**. 1982. Bioaccumulation of toxic substances during dredging. Draft Final Report of the Interagency Agreement AD-14-F-1-529-0. U.S. Environmental Protection Agency, Great Lakes National Program Office, Chicago, IL.
27. **Hoke, R.A.** and **B.L. Prater**. 1980. Relationship of percent mortality of four species of aquatic biota from 96-hour sediment bioassays of five Lake Michigan harbors and elutriate chemistry of the sediments. *Bull. Environ. Contam. Toxicol.* **25**:394–399.
28. **Laskowski-Hoke, R.A.** and **B.L. Prater**. 1981. Dredged material evaluation: Correlations between chemical and biological procedures. *J. Water Pollut. Control Fed.* **53**:1260–1262.
29. **Lee, G.F.** and **R.A. Jones**. 1982. Discussion of dredged material evaluations: Correlations between chemical and biological procedures. *J. Water Pollut. Control Fed* 54:406–407.
30. **Wentsel, R.A., A. McIntosh** and **G. Atchison**. 1977. Sublethal effects of heavy metal contaminated sediment on midge larvae (*Chironomus tentans*). *Hydrobiologia* **56**:153–156.
31. **Wentsel, R.A., A. McIntosh** and **P.C. McCafferty**. 1978. Emergence of the midge *Chironomus tentans* when exposed to heavy metal contaminated sediments. *Hydrobiologia* **57**:195–196.
32. **Shuba, P.J., H.E. Tatum** and **J.E. Carroll**. 1978. Biological assessment methods to predict the impacts of open-water disposal of dredged material. DMRP Technical Report No. D-78-50, U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.
33. **Marking, L.L., V.K. Dawson, J.L. Allen, T.D. Bills** and **J.J. Rach**. 1981. Biological activity and chemical characteristics of dredge material from ten sites on the upper Mississippi River. Summary report. U.S. Fish and Wildlife Service, LaCrosse, WI.
34. **Gannon, J.E.** and **A.M. Beeton**. 1971. Procedures for determining the effects of dredged sediments on biota-benthos viability and sediment selectivity tests. *J. Water Pollut. Control Fed.* **43**:392–398.
35. **Bailey, H.C.** and **D.H.W. Liu**. 1980. *Lumbriculus variegatus*, a benthic oligochaete, as a bioassay organism. J.C. Eaton, P.R. Parrish, and A.C. Hendricks, eds., In *Aquatic Toxicology*. ASTM STP 707. American Society for Testing and Materials, Philadelphia, PA, pp. 205–215.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.